A H-CDR2 library cassette was prepared from the oligonucleotide template CDRsH (SEQ ID NOS 11 & 12, respectively) (5'-AGGGTCTCG AGTGGGTGAGC(TRI)ATT$(TRI)_{2\text{-}3}(6)_2$(TRI)ACC(TRI)TATGCG GATAGCGTGAAAGGCCGTTTTACCATTTCACGTGATAATTCGAAAAA CACCA-3'), and primer (SEQ ID NO: 13) 5'-TGGTGTTTTTCGAATTATCA-3' for synthesis of the complementary strand, where (TRI) was a trinucleotide mixture representing all amino acids except Cys, (6) comprised the incorporation of (A/G) (A/C/G) T, resulting in the formation of 6 codons for Ala, Asn, Asp, Gly, Ser, and Thr, and the length distribution being obtained by performing one substoichiometric coupling of the (TRI) mixture during synthesis, omitting the capping step normally used in DNA synthesis.

C10

REMARKS

In view of the above amendments, it is respectfully submitted that this application is in condition for allowance. Early notice to that effect is earnestly solicited. The Examiner is invited to telephone the undersigned at the number listed below if the Examiner believes such would be helpful in advancing the application to issue.

It is believed that no fees are due with the submission of this Response. In the event any fees are required for the filing of this paper, applicants authorize the Commissioner to charge such fees to Deposit Account No. 08-1641.

Respectfully submitted,

May 29, 2002
Date

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Marked-Up Copy of Amended Paragraphs

Page 18, line 4, paragraph 2: Please amend as follows:

Figs. 2A-2G: Alignment of consensus sequences designed for each subgroup (amino acid residues are shown with their standard one-letter abbreviation). (2A-2B) (SEQ ID NOS 28-31, respectively) kappa sequences, (2C-2D) (SEQ ID NOS 32-34, respectively) lambda sequences and (2E-2G) (SEQ ID NOS 35-41, respectively), heavy chain sequences. The positions are numbered according to Kabat (1991). In order to maximize homology in the alignment, gaps (-) have been introduced in the sequence at certain positions.

Page 18, line 10, paragraph 3: Please amend as follows:

Figs. 3A-3K: Gene sequences (SEQ ID NOS 42, 44, 46 and 48, respectively) of the synthetic V kappa consensus genes. The corresponding amino acid sequences (SEQ ID NOS 43, 45, 47 and 49, respectively) (see Figs. 2A-2B) as well as the unique cleavage sites are also shown.

Page 18, line 13, paragraph 4: Please amend as follows:

Figs. 4A-4I: Gene sequences (SEQ ID NOS 50, 52 and 54, respectively) of the synthetic V lambda consensus genes. The corresponding amino acid sequences (SEQ ID NOS 51, 53 and 55, respectively) (see Figs. 2C-2D) as well as the unique cleavage sites are also shown.

Page 18, line 16, paragraph 5: Please amend as follows:

Figs. 5A-5U: Gene sequences (SEQ ID NOS 56, 58, 60, 62, 64, 66 and 68, respectively) of the synthetic V heavy chain consensus genes. The corresponding amino acid sequences (SEQ ID NOS 57, 59, 61, 63, 65, 67 and 69, respectively) (see Figs. 2E-2G) as well as the unique cleavage sites are also shown.

Page 18, line 19, paragraph 6: Please amend as follows:

Figs. 6A-6G: Oligonucleotides (SEQ ID NOS 70-164, respectively) used for construction of the consensus genes. The oligos are named according to the corresponding consensus gene, e.g. the gene Vκ1 was constructed using the six

oligonucleotides O1K1 to O1K6. The oligonucleotides used for synthesizing the genes encoding the constant domains Cκ (OCLK1 to 8) and CH1 (OCH1 to 8) are also shown.

Page 18, line 25, paragraph 7: Please amend as follows:

Figs. 7A-7D: Sequences of the synthetic genes (SEQ ID NOS 165 and 167, respectively) encoding the constant domains Cκ (7A-7B) and CH1 (7C-7D). The corresponding amino acid sequences (SEQ ID NOS 166 and 168, respectively) as well as unique cleavage sites introduced in these genes are also shown.

Page 18, line 28, paragraph 8: Please amend as follows:

Figs. 7E-7H: Functional map and sequence (SEQ ID NOS 169-170, respectively) of module M24 comprising the synthetic Cλ gene segment (huCL lambda).

Page 18, line 30, paragraph 9: Please amend as follows:

Figs. 7I-7J: Oligonucleotides (SEQ ID NOS 171-176) used for synthesis of module M24.

Page 18, line 31, paragraph 10: Please amend as follows:

Figs. 8A-8E: Sequence (SEQ ID NOS 177-178, respectively) and restriction map of the synthetic gene encoding the consensus single-chain fragment VH3-Vκ2. The signal sequence (amino acids 1 to 21) was derived from the *E. coli* phoA gene (Skerra & Plückthun, 1988). Between the phoA signal sequence and the VH3 domain, a short sequence stretch encoding 4 amino acid residues (amino acid 22 to 25) has been inserted in order to allow detection of the single-chain fragment in Western blot or ELISA using the monoclonal antibody M1 (Knappik & Plückthun, 1994). The last 6 basepairs of the sequence were introduced for cloning purposes (EcoRI site).

Page 19, line 14, paragraph 3: Please amend as follows:

Figs. 10A-10B: Sequencing results of independent clones from the initial library, translated into the corresponding amino acid sequences. (A) (SEQ ID NO: 179) Amino acid sequence of the VH3 consensus heavy chain CDR3 (position 93 to 102, Kabat numbering). (B) (SEQ ID NOS 180-191, respectively) Amino acid sequences of 12 clones of the 10-mer library. (C) (SEQ ID NOS 192-202, respectively) Amino acid sequences of 11 clones of the 15-mer library, *: single base deletion.

Page 19, line 35, paragraph 8: Please amend as follows:

Fig. 15: Sequences results of the heavy chain CDR3s of independent clones after 3 rounds of planning against FITC-BSA, translated into the corresponding amino acid sequences (SEQ ID NOS 203-218, respectively) (position 93 to 102. Kabat numbering).

Page 20, line 12, paragraph 5: Please amend as follows:

Fig. 20: Sequencing results of the heavy chain CDR3s of independent clones after 3 rounds of panning against β-estradiol-BSA, translated into the corresponding amino acid sequences (SEQ ID NOS 219-230 respectively) (position 93 to 102, Kabat numbering). One clone is derived from the 10mer library.

Page 20, line 16, paragraph 6: Please amend as follows:

Fig. 21: Sequencing results of the heavy chain CDR3s of independent clones after 3 rounds of panning against testosterone-BSA, translated into the corresponding amino acid sequences (SEQ ID NOS 231-236, respectively) (position 93 to 102, Kabat numbering).

Page 20, line 20, paragraph 7: Please amend as follows:

Fig. 22: Sequencing results of the heavy chain CDR3s of independent clones after 3 rounds of panning against lymphotoxin-β, translated into the corresponding amino acid sequences (SEQ ID NOS 237-244, respectively) (position 93 to 102, Kabat numbering). One clone comprises a 14mer CDR, presumably

introduced by incomplete coupling of the trinucleotide mixture during oligonucleotide synthesis.

Page 20, line 26, paragraph 8: Please amend as follows:

Fig. 23: Sequencing results of the heavy chain CDR3s of independent clones after 3 rounds of panning against ESL-1, translated into the corresponding amino acid sequences (SEQ ID NOS 245-256, respectively) (position 93 to 102, Kabat numbering). Two clones are derived from the 10mer library. One clone comprises a 16mer CDR, presumably introduced by chain elongation during oligonucleotide synthesis using trinucleotides.

Page 20, line 32, paragraph 9: Please amend as follows:

Fig. 24: Sequencing results of the heavy chain CDR3s of independent clones after 3 rounds of panning against BSA, translated into the corresponding amino acid sequences (SEQ ID NOS 257-262, respectively) (position 93 to 102, Kabat numbering).

Page 21, line 1, paragraph 1: Please amend as follows:

Figs. 27A-27B: Functional map and sequence (SEQ ID NO: 263) of the multi-cloning site module (MCS).

Page 21, line 2, paragraph 2: Please amend as follows:

Figs. 28A-28G: Functional map and sequence (SEQ ID NO: 264-265, respectively) of the pMCS cloning vector series.

Page 21, line 3, paragraph 3: Please amend as follows:

Figs. 29A-29B: Functional map and sequence (SEQ ID NO: 266) of the pCAL module M1 (see Figs. 26A-26D).

Page 21, line 4, paragraph 4: Please amend as follows:

Figs. 30A-30C: Functional map and sequence (SEQ ID NOS 267-268, respectively) of the pCAL module M7-III (see Figs. 26A-26D).

Page 21, line 5, paragraph 5: Please amend as follows:

Figs. 31A-31B: Functional map and sequence (SEQ ID NO: 269) of the pCAL module M9-II (see Figs. 26A-26D).

Page 21, line 6, paragraph 6: Please amend as follows:

Figs. 32A-32C: Functional map and sequence (SEQ ID NO: 270) of the pCAL module M11-II (see Figs. 26A-26D).

Page 21, line 7, paragraph 7: Please amend as follows:

Figs. 33A-33D: Functional map and sequence (SEQ ID NO: 271) of the pCAL module M14-Ext2 (see Figs. 26A-26D).

Page 21, line 9, paragraph 8: Please amend as follows:

Figs. 34A-34D: Functional map and sequence (SEQ ID NOS 272-273, respectively) of the pCAL module M17 (see Figs. 26A-26D).

Page 21, line 10, paragraph 9: Please amend as follows:

Figs. 35A-35I: Functional map and sequence (SEQ ID NOS 274-276, respectively) module vector pCAL4.

Page 21, line 11, paragraph 10: Please amend as follows:

Figs. 35J-35XXX: Functional maps and sequences (SEQ ID NOS 277-300, respectively) of additional pCAL modules (M2, M3, M7I, M7II, M8, M10II, M11II, M12, M13, M19, M20, M21, M41) and of low-copy number plasmid vectors (pCALO1 to pCALO3).

Page 21, line 14, paragraph 11: Please amend as follows:

Figs. 35YYY-35CCCC: List of oligonucleotides and primers (SEQ ID NOS 301-360, respectively) used for synthesis of pCAL vector modules.

Pag 21, line 16, paragraph 12: Please amend as follows:

Figs. 36A-36F: Functional map and sequence (SEQ ID NOS 361-362, respectively) of the β-lactamase cassette for replacement of CDRs for CDR library cloning.

Page 21, line 18, paragraph 13: Please amend as follows:

Figs. 37A-37D: Oligo and primer (SEQ ID NOS 363-367, respectively) design for Vκ CDR3 libraries.

Page 21, line 19, paragraph 14: Please amend as follows:

Figs. 38A-38D: Oligo and primer (SEQ ID NOS 368-371, respectively) design for Vλ CDR3 libraries.

Page 22, line 12, paragraph 3: Please amend as follows:

Table 4: Computation of the consensus sequence of the rearranged V kappa sequences. (A) (SEQ ID NO: 14), V kappa subgroup 1, (B) (SEQ ID NO: 15), V kappa subgroup 2, (C) (SEQ ID NO: 16), V kappa subgroup 3 and (D) (SEQ ID NO: 17), V kappa subgroup 4. The number of each amino acid found at each position is tabulated together with the statistical analysis data. (1) Amino acids are given with their standard one-letter abbreviations (and B means D or N, Z means E or Q and X means any amino acid). The statistical analysis summarizes the number of sequences found at each position (2), the number of occurrences of the most common amino acid (3), the amino acid residue which is most common at this position (4), the relative frequency of the occurrence of the most common amino acid (5) and the number of different amino acids found at each position (6).

Page 22, line 24, paragraph 4: Please amend as follows:

Table 5: Computation of the consensus sequence of the rearranged V lambda sequences. (A) (SEQ ID NO: 18), V lambda subgroup 1, (B) (SEQ ID NO: 19), V lambda subgroup 2, and (C) (SEQ ID NO: 20), V lambda subgroup 3. The number of each amino acid found at each position is tabulated together with the statistical analysis of the data. Abbreviations are the same as in Table 4.

Page 22, lin 29, paragraph 5: Please amend as follows:

Table 6: Computation of the consensus sequence of the rearranged V heavy chain sequences. (A) (SEQ ID NO: 21), V heavy chain subgroup 1A, (B) (SEQ ID NO: 22), V heavy chain subgroup 1B, (C) (SEQ ID NO: 23), V heavy chain subgroup 2, (D) (SEQ ID NO: 24), V heavy chain subgroup 3, (E) (SEQ ID NO: 25), V heavy chain subgroup 4, (F) (SEQ ID NO: 26), V heavy chain subgroup 5, and (G) (SEQ ID NO: 27), V heavy chain subgroup 6. The number of each amino acid found at each position is tabulated together with the statistical analysis of the data. Abbreviations are the same as in Table 4.

Page 29, line 1, paragraph 1: Please amend as follows:

In the case of the CDR3s, any sequence could be chosen since these CDRs were planned to be the first to be replaced by oligonucleotide libraries. In order to study the expression and folding behavior of the consensus sequences in E. coli, it would be useful to have all sequences with the same CDR3, since the influence of the CDR3s on the folding behavior would then be identical in all cases. The dummy sequences QQHYTTPP (see, for instance, positions 89-96 of SEQ ID NO: 28 and positions 88-95 of SEQ ID NO: 34) and ARWGGDGFYAMDY (positions 97-109 of SEQ ID NOS 35 & 36) were selected for the VL chains (kappa and lambda) and for the VH chains, respectively. These sequences are known to be compatible with antibody folding in E. coli (Carter et al., 1992).

Page 31, line 4, paragraph 2: Please amend as follows:

2.1 Cloning of the HuCAL VH3-Vk2 scFv Fragment

In order to test the design of the consensus genes, one randomly chosen combination of synthetic light and heavy gene (HuCAL-Vκ2 and HuCAL-VH3) was used for the construction of a single-chain antibody (scFv) fragment. Briefly, the gene segments encoding the VH3 consensus gene and the CH1 gene segment including the CDR3--framework 4 region, as well as the Vκ2 consensus gene and the Cκ gene segment including the CDR3--framework 4 region were assembled yielding the gene for the VH3-CH1 Fd fragment and the gene encoding the Vκ2-Cκ light chain, respectively. The CH1 gene segment was then replaced by an

oligonucleotide (SEQ ID NOS 2 & 3, respectively) cassette encoding a 20-mer peptide linker (SEQ ID NO: 1) with the sequence AGGGSGGGGSGGGGSGGGGS. The two oligonucleotides encoding this linker were 5'-TCAGCGGGTGGCGGTTCTGGCGGCGGTGGGAGCGGTG GCGGTGGTTCTGGCGGTGGTGGTTCCGATATCGGTCCACGTACGG-3'

and 5'-AATTCCGTACGTGGACCGATATCGGAACCACCACCGCCAGA ACCACCGCCACCGCTCCCACCGCCGCCAGAACCGCCACCCGC-3', respectively. Finally, the HuCAL-Vκ2 gene was inserted via EcoRV and BsiWI into the plasmid encoding the HuCAL-VH3-linker fusion, leading to the final gene HuCAL-VH3-Vκ2, which encoded the two consensus sequences in the single-chain format VH-linker-VL. The complete coding sequence is shown in FIG. 8.

Page 32, line 12, paragraph 1: Please amend as follows:

The CDR3 libraries of lengths 10 and 15 were generated from the PCR fragments using oligonucleotide templates (SEQ ID NOS 4 & 5, respectively) O3HCDR103T (5'-GATACGGCCGTGTATTATTGCGCGCGT $(TRI)_6$ GATTATTGGGGCCAAGGCACCCTG-3') and O3HCDR153T (5'-GATACGGCCGTGTATTATTGCGCGCGT$(TRI)_{10}$ (TTT/ATG)GAT(GTT/TAT)TGGGGCCAAGGCACCCTG-3'), and primers (SEQ ID NOS 6 & 7, respectively) O3HCDR35 (5'-GATACGGCCGTGTATTATTGC-3') and O3HCDR33 (5'-CAGGGTGCCTTGGCCCC-3'), where TRI are trinucleotide mixtures representing all amino acids without cystein, (TTT/ATG) and (GTT/TAT) are trinucleotide mixtures encoding the amino acids phenylalanine/methionine and valine/tyrosine, respectively. The potential diversity of these libraries was 4.7×10^7 and 3.4×10^{10} for 10-mer and 15-mer library, respectively. The library cassettes were first synthesized from PCR amplification of the oligo templates in the presence of both primers: 25 pmol of the oligo template O3HCDR103T or O3HCDR153T, 50 pmol each of the primers O3HCDR35 and O3HCDR33, 20 nmol of dNTP, 10x buffer and 2.5 units of Pfu DNA polymerase (Stratagene) in a total volume of 100 ml for 30 cycles (1 minute at 92°C., 1 minute at 62°C. and 1 minute at 72°C.). A hot-start procedure was used. The resulting mixtures were phenol-extracted, ethanol-precipitated and digested overnight with EagI and StyI. The vector pIG10.3-

scH3κ2cat, where the EagI-StyI fragment in the vector pIG10.3-scH3κ2 encoding the H-CDR3 was replaced by the chloramphenicol acetyltransferase gene (cat) flanked with these two sites, was similarly digested. The digested vector (35 μg) was gel-purified and ligated with 100 μg of the library cassette overnight at 16°C. The ligation mixtures were isopropanol precipitated, air-dried and the pellets were redissolved in 100 ml of ddH2O. The ligation was mixed with 1 ml of freshly prepared electrocompetent XL1 Blue on ice. 20 rounds of electroporation were performed and the transformants were diluted in SOC medium, shaken at 37°C. for 30 minutes and plated out on large LB plates (Amp/Tet/Glucose)

Page 41, line 19, paragraphs 2 and 3: Please amend as follows:

A L-CDR3 library cassette was prepared from the oligonucleotide (SEQ ID NO: 9) template CDR3L (5'-TGGAAGCTGAAGACGTGGGCGTGTATTATT GCCAGCAG(TR5)$(TRI)_4$CCG(TRI)TTTGGCCAGGGTACGAAAGTT-3') and primer (SEQ ID NO: 10) 5'-AATTTCGTACCCTGGCC-3' for synthesis of the complementary strand, where (TRI) was a trinucleotide mixture representing all amino acids except Cys, (TR5) comprised a trinucleotide mixture representing the 5 codons for Ala, Arg, His, Ser, and Tyr.

A H-CDR2 library cassette was prepared from the oligonucleotide template CDRsH (SEQ ID NOS 11 & 12, respectively) (5'-AGGGTCTCG AGTGGGTGAGC(TRI)ATT$(TRI)_{2\text{-}3}(6)_2$(TRI)ACC(TRI)TATGCG GATAGCGTGAAAGGCCGTTTTACCATTTCACGTGATAATTCGAAAAA CACCA-3'), and primer (SEQ ID NO: 13) 5'-TGGTGTTTTTCGAATTATCA-3' for synthesis of the complementary strand, where (TRI) was a trinucleotide mixture representing all amino acids except Cys, (6) comprised the incorporation of (A/G) (A/C/G) T, resulting in the formation of 6 codons for Ala, Asn, Asp, Gly, Ser, and Thr, and the length distribution being obtained by performing one substoichiometric coupling of the (TRI) mixture during synthesis, omitting the capping step normally used in DNA synthesis.